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Horse gram (*Macrotyloma uniflorum*) a traditional food medicine protects the kidney from the cisplatin-induced nephrotoxicity in albino rats

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The nutritional seeds from *Macrotyloma uniflorum* (MU) were studied for their unexplored beneficial effect in kidney diseases. Thus, the present study was designed to investigate the MU seed for its nephroprotective efficacy against cisplatin-induced nephrotoxicity in an animal model. The nephroprotective potential for the *Macrotyloma uniflorum* seed extract (MUSE) was evaluated in albino rats (250 and 500 mg/kg; p.o.) for 10 days. At the end of the study, the nephroprotective effect was assessed based on the levels of various biochemical parameters of serum, urine, and kidney tissue. The GC-MS analyses of extract confirmed the antioxidant phytochemical constituents responsible for the nephroprotective activity. The MUSE treatment at 250 and 500 mg/kg p.o. showed a significant reduction in the levels of blood urea nitrogen ($p<0.05$), serum creatinine ($p<0.01$), serum urea ($p<0.001$), restore the urine volume ($p<0.01$), and urine creatinine ($p<0.01$) as compared to the negative control (cisplatin-injected rats). Subsequently, the abnormal conditions of creatinine clearance ($p<0.05$) and serum albumin ($p<0.05$) were restored to normal. For respective doses of 250 and 500 mg/kg p.o, the superoxide dismutase activity increased ($p<0.05$) to 82.91 and 98.8 U/g whilst the glutathione level significantly increased ($p<0.01$) to 36.24 and 46.17 µg/mg. Similarly, the catalase activity was significantly improved ($p<0.001$) to 485.9 and 573.5 µM/g with a significant reduction ($p<0.001$) in the level of the malondialdehyde to 84.56 and 68.56 nM/mg, respectively for low and high doses. The histopathology of kidney tissues confirmed the pronounced nephroprotection effect of MUSE against the cisplatin-induced injuries like tubular necrosis, hyaline cast, and vascular congestions. The major constituents found in GC-MS were inositol, ethyl alpha-d-glucopyranoside, n- hexadecanoic acid, 9, 12-octadecadienoic aci, 3-beta-stigmast-5-en-3-ol, Vitamin E, and stigmasterol. The results indicate the promising nephroprotective potential of *Macrotyloma uniflorum* seeds in nephrotoxicity induced rats, and the efficacy was comparable with vitamin E.

Keywords: Cisplatin, *Dolichos uniflorus* Lam Kidney, *Macrotyloma uniflorum* seeds, Nephroprotective, Nephrotoxicity

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The "Food is medicine and medicine is food" concept is very supportive for reducing the current global disease burden. Nowadays “food as medicine” approach has gained popularity, and even the public of the developed nations have adopted the practice as an adjuvant in the management of many metabolic and nutritional complications such as chronic kidney disease (CKD), obesity and diabetes[1].

Horse gram (Source: *Macrotyloma uniflorum* (MU); Syn*: Dolichos uniflorus* Lam*;* Binomial name*: Macrotyloma uniflorum* Lam. Verdc.; Family: Fabaceae (alt. Leguminosae) is known as kollu (Tamil), ulavalu (Telugu), and kulthi (Hindi). It is widely cultivated and consumed in India since ancient times, and native to the South-east Asian subcontinent and tropical Africa. According to Ayurveda, horse gram has been used as traditional food medicine in kidney stones, and hemorrrhoids for its unexceptional nutritional and therapeutic potential. In folk medicine, horse gram is used in hypercholesterolemia, jaundice, rheumatoid fever, leucorrhoea, bleeding piles and diarrhea[2]. The seeds are a rich source of iron, calcium, molybdenum, and proteins. The major chemical constituents present in the MU seeds include trypsin inhibitors and natural phenols like 3,4-dihydroxy benzoic acid, 4-hydroxy benzoic acid, vanillic acid, caffeic acid, p-cumaric acid, ferulic acid, syringic acid, and sinapic acid[3].

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In the literature survey, MU seeds are reported to be useful for postprandial hyperglycemia, they attenuate the insulin resistance by inhibiting tyrosine phosphatase 1- beta enzyme[4]. The leaves and green pods of the plant were used in vitamin C and vitamin A deficiencies, respectively. And the seeds have been reported as a more potent diuretic than acetazolamide[5]. In addition, the whole plant of MU was studied for antihypercholesterolemic and antimicrobial activities[6-9], and similarly, the leaves studied for antiobese[10,11] and antihelminthic[12,13]. antidiabetic[14,15], anticholilithiatic[16], antihistaminic[17], antiulcer[18], antiobese[19], antiurolithiatic[20], diuretic[21], haemolytic[22], hepatoprotective[23], alpha-amylase inhibitor[24], proteinase inhibitor[25], and ACE-1 inhibitor[26], also antinephrolithiatic and antioxidative efficacies were studied using *in vitro* models[27]. As of now, there is no available report on nephroprotection. In the present study, we investigated the MU seeds extract nephroprotective efficacy in cisplatin-induced nephrotoxicity model. We estimated the levels of kidney function markers, oxidative stress markers, and followed by histopathology of kidney tissues, and GC-MS for the extract were carried.

Material and Methods

Chemicals and instrumentation

Cisplatin was obtained as gift sample from Mylan Pvt. Ltd, India and the standard Vitamin E sample was procured from Merck, Mumbai, India. All reagents and solvents used in this study were analytical reagents (AR Grade) obtained from Merck, Inc., including EDTA, quercetin, aluminium chloride, xanthine oxidase, 5,5-dithiobis-2-nitrobenzoic acid (DTMB), sodium chloride, phosphate buffer, hydrogen peroxide, malondialdehyde, thiobarbituric acid, tris-hydrochloride, petroleum ether, acetone and chloroform. Glasswares used were calibrated as Glass A as per Indian Pharmacopoeia. The UV-Visible spectrophotometer (UV 3000+ Lab India) was used in absorbance measurement. The GC-MS analysis was carried out on GC-MS SHIMADZU GC-2010 (short (< 5 m) sin column, helium as carrier gas.

Plant material

The seeds of *Macrotyloma uniflorum* (MU) were collected from the cultivating areas (14.5968° N, 77.6779° E) of Anantapur district of Andhra Pradesh, India. The seeds were botanically authenticated by the Department of Botany, Sri Krishnadevaraya University, Anantapur, Andhra Pradesh, India (Voucher no: 432/2018), and they were carefully processed for the removal of foreign matters, shade dried and then powdered to coarse.

Preparation of extract

A quantity of 150 g of MU seed powder was soaked in 500 mL of petroleum ether, allowed for 24 h, and then filtered for removing the fatty matter. The marc was dried at room temperature and re-extracted with 500 mL of acetone under the dark condition at 25±2°C for 72 h. The resultant mix was filtered through Whatmann filter paper No. 4 and then the solvent was removed under the reduced pressure to obtain the solid mass of MU seed extract (MUSE).

Phytochemical analysis

A suitable quantity of MUSE was dissolved in ethanol to obtain the concentration of 10% *w/v.* This extract solution was used in the phytochemical tests for the presence of flavonoids, carbohydrates, proteins, fat, steroids, tannins, terpenes, glycosides, alkaloids and saponins[28]. Finally, the total flavonoid content was determined by aluminum chloride based colorimetric analysis[29] using quercetin as standard to construct a calibration curve in the range between 5-500 µg/mL.

Animals

A total of 30 male albino rats (150-200 g) were procured from Sri Venkateshwara enterprise Ltd, Bangalore, India and housed at ambient temperature of 22±1°C and relative humidity of 55±5%, and exposed to 12 h/12 h light-dark cycle. Animals were allowed to commercial rat pellets diet (VRK nutrients) and water ad libitum. The study protocol was duly approved by the institutional animal ethical committee (IAEC) of Raghavendra Institute of Pharmaceutical Education and Research, AP, India (Protocol approved No. (878/ac/05/006/2016) as per the stipulated guidelines of Committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Experimental design

After 7 days of acclimatization, the albino rats were divided (n=6) into five groups (I, II, III, IV and V). The group I served as normal control (received 0.9% saline as a vehicle) while the group II was negative control (received cisplatin only on 7th day). Group III was assigned as a positive control (treated with Vitamin E; 100 mg/kg body weight. p.o) and the group IV and V animals were assigned as test

groups received MUSE orally at the doses of 250 and 500 mg/kg; p.o., respectively for 10 days. On the 7th day, except group I, the remaining groups (II, III, IV and V) were subjected to nephrotoxicity induction by single-dose cisplatin injection (7 mg/kg; i.p.) and further, the MUSE treatment was continued upto 10th day.

Sample collection and tissue preparation

On the 10th day, the rats were placed in metabolic cage for 24 h urine collection. The collected urine sample was used to determine the urinary volume (mL/24 h), urine creatinine (mg/dL) and creatinine clearance (mL/h/100 g body weight). Further, the animals were sacrificed through anaesthetization and then followed by cervical dislocations. The collected blood samples using cardiac puncture were used for the estimation of serum blood urea (BUN), serum creatinine (SC), serum albumin (SA) and serum urea (SU). The results are shown in Table 1 and Figure 1 and Figure 2. Soon after the blood collection, kidneys were removed, washed with physiological saline solutions (0.9% w/v), then buffered and stored at -80°C until further analyses. A part of kidney tissue (1:8) was homogenized in 0.9% saline and centrifuged for 15 min. The transparent supernatant layer was removed and used for analyses of oxidative stress markers including superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and malondialdehyde (MDA). The results are presented in Table 2 and Figure 3.

Biochemical analyses of blood and urine

The urine creatinine (UC), serum creatinine (SC), serum albumin (SA), serum urea (SU) and blood urea nitrogen (BUN) were estimated using colorimetric assay as per the procedure reported in the previous literature[30,31]. The results are shown in Table 1 and Figure 1 and Figure 2.

Estimation of biomarkers in oxidative stress in kidney tissue

The superoxide dismutase (SOD) activity in kidney tissue of rats was measured using xanthine oxidase reagent[32]. The levels of reduced glutathione (GSH) was colorimetrically determined by 5, 5- dithiobis 2-nitrobenzoic acid (DTMB) method[33]. The catalase activity (CAT) was estimated by the hydrogen peroxide method. The malondialdehyde (MDA) level in tissue was determined by the spectrophotometric method as per the report[34]. The results are shown in Table 2 and Figure 3.

Histopathological examinations

The collected kidney tissue was washed with physiological saline solution (0.9% w/v NaCl) and fixed in formalin (10%). Further they were processed, embedded in paraffin, and then micro-sectioned for histopathological studies. The sectioned tissues were stained with haematoxylin and eosin prior to observation under the light microscope and the obtained images are shown in Figure 4.

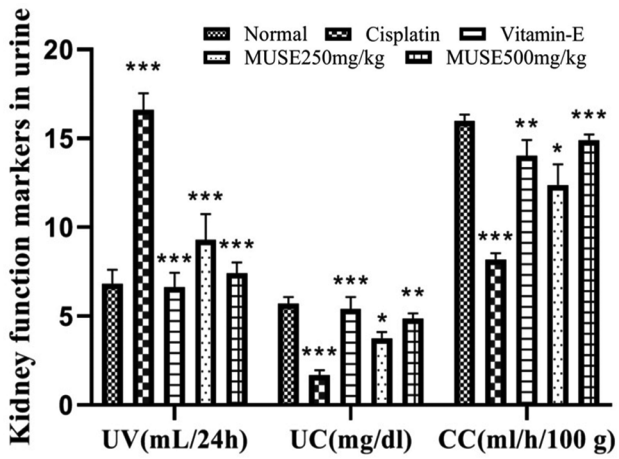

Fig. 1 — Effect of MUSE extract on renal function parameters in cisplatin-induced nephrotoxicity in rats (*p<0.05, **p<0.01, ***p<0.001)

Table 1 — Effect of *Macrotyloma uniflorum* seeds extracts on kidney function markers in serum and urine of normal and experimental rats

Group	Kidney function markers in rat serum				Kidney function markers in rat urine		
	BUN	SC	SA	SU	UV	UC	CC
Normal	16.84±1.732	0.6900±0.1136	3.530±0.1795	37.67±1.574	6.800± 0.8083	5.71± 0.357	15.98±0.3688
Cisplatin	78.50±9.23***^{a}	3.433±0.1764***^{a}	1.021±0.4284***^{a}	115.3±2.792***^{a}	16.60±0.9452***^{b}	1.67± 0.294***^{a}	8.193±0.3505***^{a}
Vitamin-E	41.86±4.437 *^{b}	1.237±0.2354***^{b}	3.147±0.09399***^{b}	55.29±2.738 ***^{b}	6.630±0.8133***^{b}	5.40± 0.670***^{b}	14.02±0.8875**^{b}
MUSE 250 mg/kg	42.14±5.280*^{c}	1.930±0.3412**^{c}	2.399±0.2568*^{b}	72.37±6.049***^{b}	9.297±1.449**^{b}	3.75± 0.360*^{b}	12.38±1.165*^{b}
MUSE 500 mg/kg	38.49±7.276**^{b}	1.053±0.1415***^{b}	2.600±0.1514 *^{b}	57.23±4.271***^{b}	7.420±0.6048***^{b}	4.85± 0.300**^{b}	14.90±0.3267***^{b}

BUN: Serum blood urea nitrogen (mg/dL); SC: Serum creatinine (mg/dL); SA: Serum albumin (g/dL); SU: Serum urea (mg/dL); UV: Urinary Volume (mL/24 h); UC: Urine creatinine (mg/dL); CC: Creatinine clearance (mL /h/100 g body weight). * p<0.05, ** p<0.01, ***p <0.001. Values are expressed as mean ± SEM (n=6) for six animals in the group; a: considered statistically significant as compared to control group; b: considered statistically significant as compared to cisplatin group.

Table 2 Effect of *Macrotyloma uniflorum* seeds extract on biomarkers of Oxidative stress in kidney tissues of normal and experimental rats

Group	SOD	CAT	GSH	MDA
Normal	99.61±11.56	614.2±20.19	49.26±2.148	45.95±3.462
Cisplatin	31.33±3.79***[a]	180.3±11.51***[a]	23.06±1.633***[a]	167.0±3.644***[a]
Vitamin E	97.49±6.394**[b]	544.3±34.54***[b]	40.65±0.8412***[b]	65.23±2.983 ***[b]
MUSE 250 mg/kg,	82.91±6.412*[b]	485.9±20.32***[b]	36.24±2.303**[b]	84.56±4.746 ***[b]
MUSE 500 mg/kg	98.80±11.75**[b]	573.5±17.65***[b]	46.17±2.301***[b]	68.56±9.532 ***[b]

SOD: Superoxide dismutase (U/g of tissue); CAT: Catalase (μM of H_2O_2 consumed/g of tissue); GSH: reduced glutathione (μg /mg of protein); MDA: melondialdehyde (nM /mg of protein). * p<0.05, ** p<0.01, ***p<0.001. Values are expressed as mean ± SEM (n=6) for six animals in the group. a: considered statistically significant as compared to control group; b: considered statistically significant as compared to cisplatin group.

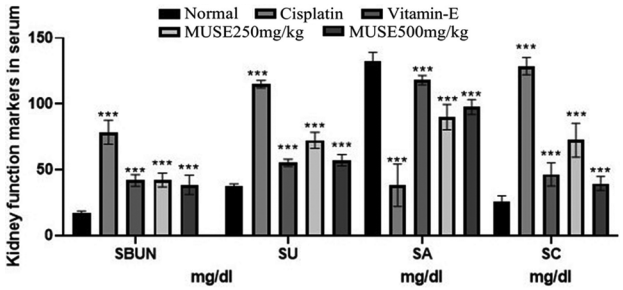

Fig. 2 — Effect of MUSE extract on serum biomarkers of renal function in cisplatin-induced nephrotoxicity in rats (*p<0.05, ** p<0.01, ***p<0.001)

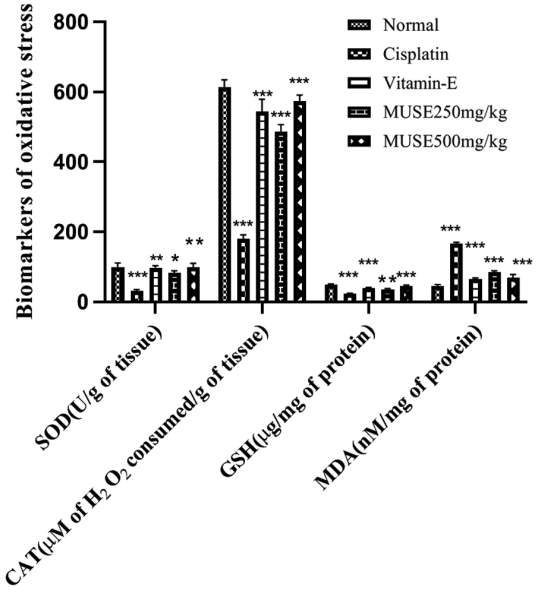

Fig. 3 — Effect of MUSE extract on tissue (kidney) antioxidant status in cisplatin-induced nephrotoxicity in rats (*p<0.05, ** p<0.01, ***p<0.001)

GC-MS analysis

The GC-MS analysis for the MUSE was performed using short (<5 m) sin column a constant mass flow rate helium at 1.5 mL/min. Initial oven temperature was at 70°C, and then it was gradually reached up to 300°C 35 min. The sample quantity of 4.0 μL was injected for analysis. The sample injector temperature was set at 260°C whilst the split ratio of injector was set at 1:20. The ionization was done with 70 eV. The mass spectrum was scanned for the mass range from 40 to 1000 *m/z* for 35 min. The m/z value of the peak was used to identify the compounds (Fig. 5).

Statistical analysis

The experimental data were statistically treated using Prism 6 software and they are expressed as mean±SEM. The data were analyzed using a one-way analysis of variance (ANOVA). The p-value less than 0.05 was inferred as statistically significant.

Results

Phytochemical analyses of MU seed extract (MUSE)

The yield (%) of the acetone extract was 8.99% *w/w* and it appeared as pale brown solid mass with a pleasant odour. The identification test for the phytochemical constituents of MUSE revealed the presence of alkaloids, glycosides, carbohydrates, proteins and amino acids, phenols and flavonoids. The total flavonoid content in MUSE was spectrophotometrically estimated from the quercetin calibration curve (R^2 = 0.9994; 5-500 μg/mL) using aluminium chloride reagent[35]. It was found to be 12.70 μg/mL quercetin equivalents.

Animal treatment

In the 10 days of treatment schedule, on 7th day, except group I (normal control), remaining groups (II, III, IV and V) were induced nephrotoxicity using cisplatin (7 mg/kg; i.p.). At the end of treatment schedule, urine and serum samples were subjected to the estimation of various kidney function parameters (Table 1), in addition oxidative stress markers were also determined in kidney tissues (Table 2). The comparison of the estimated parameters between group I and group II revealed the induction

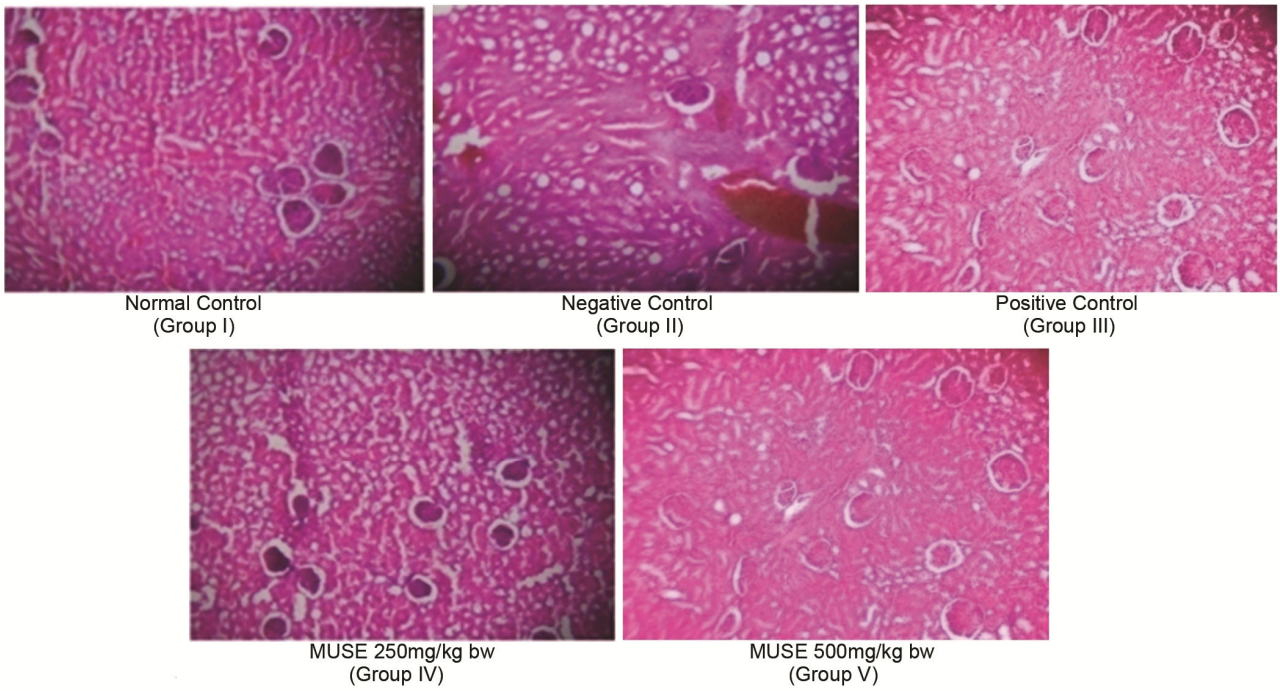

Fig. 4 — Histopathology of kidney tissues of MUSE extract treated nephrotoxicity induced rats and normal rats

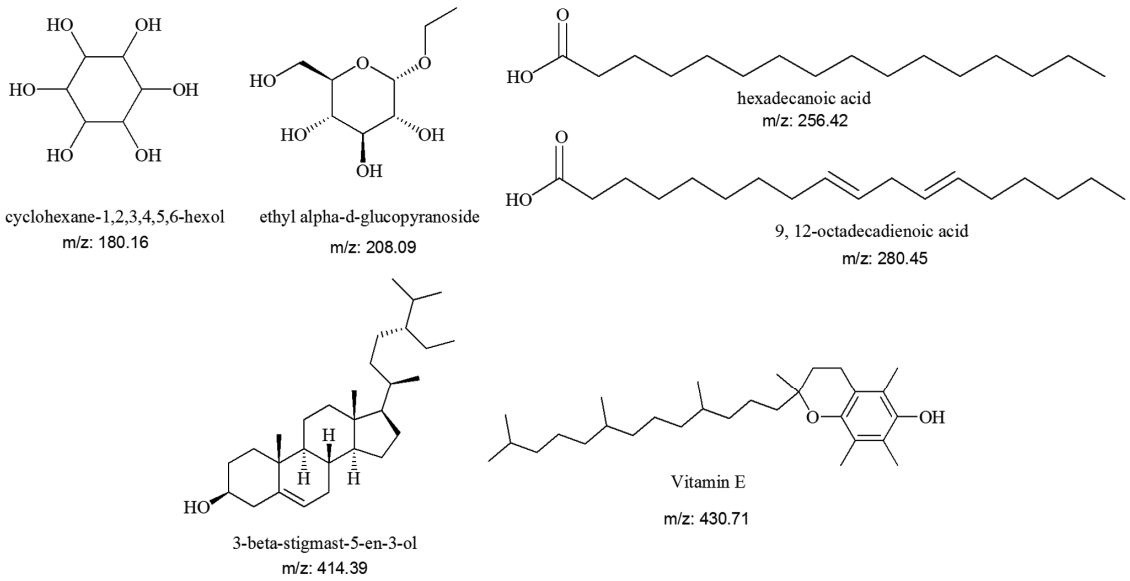

Fig. 5 — Chemical structures of major constituent found in MU seed extract by GC-MS analysis

nephrotoxicity. The data of test groups (groups IV and V) comparatively evaluated with groups III, II and I to reveal the nephroprotective effect of MUSE treatment. The p-value less than 0.05 was used to denote the statistical significance.

Effect MUSE extract on urine and serum markers

In Table 1, an injection of cisplatin (7 mg/kg; i.p.) caused a marked reduction in renal function, as characterized in group II by the significant ($p<0.001$) increase in BUN (Blood Urea Nitrogen), serum creatinine (SC) and serum urea (SU). This renal impairment was also supported by significant ($p< 0.001$) reduction in serum albumin (SA) levels in group II as compared with group I. Besides, the cisplatin injection significantly increased the urine volume (UV) but decreased in urinary creatinine (UC) and creatinine clearance (CC). Thus, these observations suggest that

the cisplatin injection impaired the kidney functions through induction of nephrotoxicity[36,37].

The MUSE-treated groups IV and V at respective doses of 250 and 500 mg/kg p.o., showed a significant decrease in the serum levels of BUN from 78.50±9.237 mg/dL (group II) to 42.14±5.280 mg/dL (group IV) and 38.49±7.276 mg/dL (group V), serum creatinine (SC) from 3.43±0.17 mg/dL (group II) to 1.93±0.34 mg/dL (group IV) and 1.05±0.14 mg/dL (group V), serum urea (SU) from 115.3±2.792 mg/dL (group II) to 72.37±6.049 mg/dL (group IV) and 57.23±4.271 mg/dL (group V), serum albumin (SA) level from 1.021±0.4284 mg/dL (group II) to 2.399±0.2568 mg/dL (group IV) and 2.600±0.1514 mg/dL (group V). Furthermore MUSE treatment significantly increased urinary creatinine (UC) from 1.67±0.294 mg/dL (group II) to 3.75±0.360 mg/dL (group IV) and 4.85±0.300 mg/dL (group V), creatinine clearance (CC) from 8.193±0.3505 mL/h/100 g body weight (group II) to 12.38±1.165 mL/h/100 g body weight (250 group IV) and 14.90±0.3267 mL/h/100 g body weight (group V) and restored the urinary volume (UV) to a normal as same as observed in group III (positive control with vitamin E). Hence, we inferred the positive nephroprotective effect of MUSE at the doses of 250 and 500 mg/kg p.o.

Effect of MUSE extracts on biomarkers of oxidative stress in kidneys

MUSE treatment restored the levels of renal antioxidant enzyme in test groups (group IV and V). The observed change in the markers was as follows: the superoxide dismutase (SOD) in U/g from 31.33±3.791 (group II) to 82.91±6.412 (250 mg/kg; p.o.) and to 98.80±11.75 (500 mg/kg p.o.), the reduced glutathione (GSH) in µg/mg from 23.06±1.633 (group II) to 36.24±2.303 (250 mg/kg p.o.) and to 46.17±2.301 (500 mg/kg; p.o.), and the catalase (CAT) activity in U/mg from 180.3±11.51 to 485.9±20.32 (250 mg/kg; p.o.) and to 573.5±17.65 (500 mg/kg p.o.). The level of MDA (melondialdehyde) in nmol/ml was reduced from 167.0±3.644 (group II) to 84.56±4.746 (250 mg/kg; p.o.) and to 68.56±9.532 (500 mg/kg; p.o.) was recorded. The results are shown in Table 2. In group II, there was significant ($p<0.001$) depletion of antioxidants with subsequent increase ($p<0.001$) in the level of MDA was observed. The MUSE treatment significantly restored the antioxidant status as demonstrated by the increased level of GSH and, SOD and CAT activities as similar to Vitamin E (group III). This revealed that the MUSE treatment alleviated the free radical-mediated injury through up-regulation of antioxidant enzyme activities in kidney tissues[38].

Histopathological studies of kidney tissue

The normal group animals had well-preserved renal architecture, indicating normal renal function. In the cisplatin-induced group, mesangial cells were damaged, and inflammatory cells infiltrated the interstitium, resulting in necrotic lesions. In the vitamin E-treated group, there was evidence of mesangial cell regrowth and negligible interstitial infiltration. The photomicrographs of the MUSE-treated groups demonstrate a reduction in inflammatory cell infiltration and reversal of kidney damage, suggesting that the test extracts had a nephroprotective effect (Fig. 4).

GC-MS analysis

The m/z value of peaks found in the GC-MS spectra indicate the presence of the compounds like, mame inositol, n- hexadecanoic acid, 9, 12-octadecadienoic acid, ethyl alpha-d-glucopyranoside, 3-beta-stigmast-5-en-3-ol, stigmasterol and Vitamin E (Fig. 5).

Discussion

In this study, cisplatin-induced nephrotoxic rat model was chosen due to its high clinical relevance and acceptable reproducibility. In the earlier days, it was used to induce acute nephrotoxicity only, but eventually this model has gained much attention to develop chronic kidney injury. This model has proven the effect of cisplatin on tissue histology as function of the rat kidney with single or multiple dose administrations of cisplatin[39]. The function of the inflammatory cells in the development of cisplatin-induced nephrotoxicity has been well studied, however the selective inhibitory effect of nephroprotective agent on either neutrophils or nuclear killer cells will not protect the kidney from the nephrotoxic effect of cisplatin. In fact, if an agent inhibits both neutrophils and nuclear killer cells, it can certainly diminish the acute nephrotoxicity effect of cisplatin[40]. In fact, the dose of the cisplatin determines the magnitude of toxic effect as either cell injury or cell death. Hence, the dose of the cisplatin was optimized at 7 mg/kg i.p., suitable for cell injury, and this model is quite relevant for adapting in drug discovery strategies because cisplatin can upregulate various receptors and leukocyte populations[41]. The MUSE extract was found to be considerably rich in flavonoids and phenolic acids. From the existing knowledge on flavonoids, we presume that the flavonoids present in MUSE extract could be the inhibitors of both neutrophils and nuclear

killer cells, and they interfere in the upregulation of receptors in a biological system.

Nephrotoxicity associated with cisplatin injection was clearly evident by the elevation in serum blood urea nitrogen, serum creatinine and decreased serum albumin, they reflect the dysfunction of glomerular filtration. Similarly, renal damage by cisplatin was also reflected by increase in urine volume (UV) because of impairment of reabsorption process in the renal tubules, decreases in urine creatinine (UC) and creatinine clearance (CC). The mechanism of renal damage by cisplatin was well reported and has consequences of renal inflammation, oxidative stress and renal apoptosis. Whereas MUSE extract demonstrated protective effect against the cisplatin-induced renal damage and it is observed by restoration serum blood urea nitrogen, creatinine, albumin and urine biochemical profiles such as urine volume, urine creatinine (UC) and creatinine clearance (CC). The degree of protective effect observed at both MUSE dosages (250 and 500 mg/kg) and the changes were in line with previous reports[42].

The MUSE dose at 250 mg/kg p.o. reduced ($p<0.05$) the BUN level from 78.50 (group II) to 42.14 mg/dL (group IV). A similar effect was recorded with group V that received the dose of 500 mg /kg; p.o ($p<0.01$). For group IV (250 mg/kg; p.o), the elevated level of SC (3.43) and SU (115.3) found in group II was significantly reduced to 1.93 ($p<0.01$) and 2.93 mg/dL ($p<0.05$), respectively. Thus, the reduction in the levels of BUN, SC, and SU was in good agreement with group III (Vitamin E). The reduced level of SA in group II due to nephrotoxicity was considerably ($p<0.05$) elevated to 2.399 and 2.600, respectively, by the MUSE doses of 250 and 500 mg/kg; p.o. The one sample t-test for the nephroprotective effect of MUSE seed extract on the restoration of serum kidney markers were quite good with acceptable statistical significance ($p<0.05$).The Figure 1 and Figure 2 shows that the extract showed activity with statistical significance of $p>0.001$.

There were three kidney function markers in urine that were estimated including urine volume (UV), urine creatinine (UC) and creatinine clearance (CC). The respective normal levels of UV, UC and CC found in group I animals There were three kidney function markers in urine that were estimated including urine volume (UV), urine creatinine (UC) and creatinine clearance (CC). The single-dose cisplatin injection caused the elevated levels of UV and UC and reduced the CC value in group II. The group III (Vitamin E) demonstrated the significant effect on the restoration of the above markers to normal value with $p<0.001$ for both UV and UC, and $p<0.01$ for CC. The MUSE extract at 250 and 500 mg/kg p.o., respectively to group IV and V, significantly reduced the elevated level of UV ($p<0.01$) and UC ($p<0.01$) to normal level as similar to vitamin E. Overall our observation is suggestive of the promising restoration effect of MUSE extract on UV, UC and CC parameters and is comparable to the effect produced by vitamin E at 10 mg/kg; p.o ($p<0.001$).

Following that, the levels of various antioxidant enzymes in the kidney homogenates of all animal groups were evaluated. According to our findings, a single dose of cisplatin causes the generation of reactive oxygen species, which leads to a reduction in anti-oxidant enzyme status (SOD, CAT, and GSH) and an increase in lipid peroxidation products such malondialdehyde. This observation supports previous findings that cisplatin-induced nephrotoxicity is mostly caused by reactive oxygen species (ROS). The antioxidant enzyme assay demonstrated that vitamin E administration increased anti-oxidant status and reduced malondialdehyde concentration. Vitamin E is a well-known antioxidant molecule. MUSE extracts improve antioxidant status in a dose-dependent way, implying that particular phytochemicals with putative antioxidant activity may be responsible for the improved antioxidant status[43].

In histopathology, cisplatin-induced nephrotoxicity probably suggested the structural abnormalities such as presence of coagulative necrosis, degeneration of renal tubules, vascular congestion and presence of hyaline casts. The above structural features were reduced and regain the normal feature of kidney by MUSE at dose dependently but at 500 mg/kg observed more protective effect

The GC-MS analysis of MUSE extract confirmed the presence of bioactive molecules such as n-hexadecanoic acid, stigmasterol, (3β)-stigmast-5-en-3-ol, vitamin and flavonoids (Fig. 5). This report was concurrent with an earlier report by Das *et al*[44].

Based on the proven antioxidant and anti-inflammatory potential of these compounds, we presume that the promising nephroprotective activity of the MUSE in rat may be due to the synergistic and cumulative antioxidant potential of the constituents[45]. Thus MUSE demonstrated the promising nephroprotection against cisplatin-induced nephrotoxicity in rats.

Conclusion

This study confirmed that *Macrotyloma uniflorum* seeds extract significantly improved the biochemical and histological alterations induced by cisplatin nephrotoxicity, thus the promising nephroprotective property of seeds was proven in rats. Due to the nutritional value of the seeds, they are free from toxicity and they can be conveniently used as neuroprotective agents. The postulated mechanisms for the nephroprotective effect include amelioration of lipid peroxidation induced by cisplatin and activation of defence mechanisms.

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Conflicts of Interest

Authors declare that there is no conflict of interest.

Author's Contributions

AS and VK equally contributed in conceptualization of the study and drafted the manuscript and performed statistical analysis. R J was involved in the animal treatment and data collection. SM and PL were involved in the extraction process and phytochemical analyses. PR and KSR elaborated the manuscript and structured the discussion. All authors agreed to this submission after careful reading and approval.

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